Utilizing *C. elegans* to model tauopathy

Presented by Cheong Maria Yu

In partial fulfillment of the requirements for graduation with the Dean's Scholars Honors Degree in Biology

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Abstract

Many neurodegenerative diseases are characterized by abnormal accumulation of proteins, including tauopathies such as Alzheimer's disease (AD). AD is the most common form of dementia that is characterized by cognitive losses and neurodegeneration. Due to the presence of genes homologous to human genes known to be involved in AD development, the nematode *Caenorhabditis elegans* can be used to model neurodegenerative diseases such as AD. Here I contribute to the available nematode models by creating a new multi-copy overexpression tau model, generating the tools needed to create a single-copy overexpression tau model. I made and characterized a transgenic model expressing an extra-chromosomal array of a mutant form of tau (*tau-P301L)* driven by the promoter for the worm homolog of *tau*. Like a previous tau overexpression strain, this strain demonstrated impaired locomotion and decreased egg-laying relative to wild-type controls. However, the current model shows an adult-onset rather than larval-onset of behavioral impairments. A single-copy integration of the tau mutant, for which I prepared the constructs, will help to determine if the later onset in the current model is dose or location sensitive. Adult onset of impairments is an improvement over earlier onset in that it is easier to explore neurodegeneration outside of the context of development.

1. Introduction

1.1 Age-related neurodegenerative disease: An overview

Several neurodegenerative diseases have been associated with abnormal aggregation of proteins, including Parkinson's disease and Alzheimer's disease (AD) [1, 2]. The most common form of dementia [3, 4], Alzheimer's disease (AD) remains an unsolved mystery. With the expanding size of the elderly population due to prolonged lifetimes and the aging baby-boomer generation, the prevalence of AD is predicted to increase rapidly. AD patients suffer from gradual cognitive decline, including memory and language deficits. AD is characterized by two main hallmarks: Aβ plaques, formed from cleavage products of amyloid precursor protein (APP), and neurofibrillary tangles (NFTs), composed of hyperphosphorylated tau protein [3, 4]. However, the roles of Aβ plaques and NFTs in the development of AD are not yet clear.

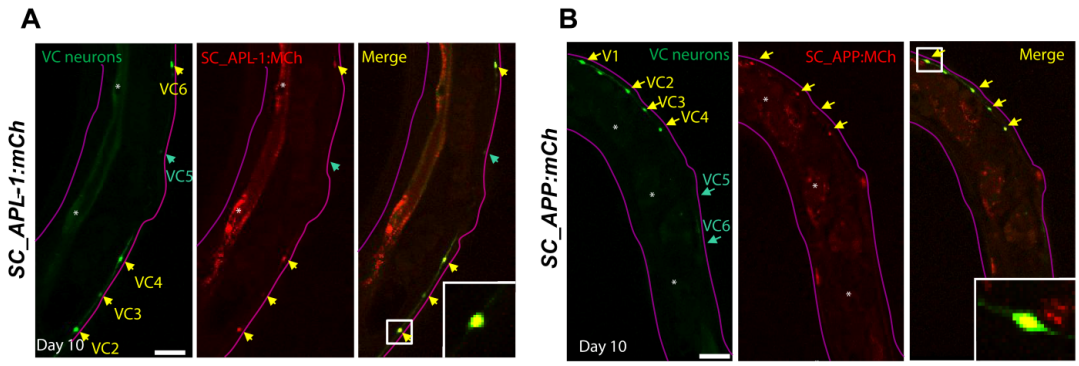

Figure 1. Use of fluorescent reporters to view neurons and APP and apl-1 accumulation via confocal microscopy in *C. elegans* (image from [13] Crisp *et al.,* 2012).

1.2 Tau pathology

Several neurodegenerative diseases, collectively called "tauopathies," have been linked to abnormalities in tau, a microtubule-associated protein involved in stabilizing the microtubules within a neuron [5, 6], including frontotemporal dementia with Parkinsonism [7] and Alzheimer's disease [1, 2]. Although the microtubule network appears to be intact even with abnormal tau [6, 8], excess levels of tau appear to negatively affect functions associated with the neuron's microtubule structure, such as vesicular transport, including transport of APP [8], which offers a possible relationship between tau and APP pathology in Alzheimer's disease. Furthermore, mutations in *tau* have been identified that result in deposits of tau often characterized by rope-like or twisted filaments, and the associated dementia may be linked to hyperphosphorylation of the mutant tau [9].

1.3 *Caenorhabditis elegans* as a model for tauopathy

With the incidence of AD predicted to increase rapidly [10], it is necessary to find ways to explore age-related degeneration on a more efficient time-scale. Due to short developmental cycles, rapid aging studies are made possible using *C. elegans*. Additionally, AD is initially characterized by degeneration of cholinergic neurons. *C. elegans* have many cholinergic neurons involved in a wide variety of functions, including locomotion and egg-laying [11] and, due to transparent bodies, neurodegeneration and localization of proteins are observable through the use of fluorescent labeling (Figure 1). Furthermore, genes homologous to those associated with AD in humans, specifically *APP* and *tau*, are present in the worm [12], making this animal an appropriate model for exploring the role of these genes in AD etiology.

1.4 Current *C. elegans* models of tauopathy

Current nematode models of AD have been created via genetic manipulation by way of microinjection of desired genes. These genes may be expressed as part of an extrachromosomal array or they may be integrated into the genome. Current nematode models of tauopathy, which includes a number of dementias including AD, express a multi-copy integrated array of both wild-type and hyperphosphorylation-prone mutant human *tau* driven by a pan-neuronal promoter [7]. These models demonstrate a number of behavioral deficits, including uncoordinated locomotion and

reduced egg-laying, and neurodegeneration [7]. However, since the observed phenotype of this model may be due to the overwhelming amount of tau expressed in the worm model, a model expressing a single copy of *tau* might lend more insight into tau-specific phenotypes. Making this model expressing *tau* driven by an endogenous promoter was attempted in this study using MosI-mediated single-copy insertion [14].

***C. elegans* Strains**			
Name	**Genotype**	**Description**	**Source**
N2	WT	wild-type strain	CGC
EG4322 (unc-119)	*ttTi5605 II; unc-119(ed3) III*	unc, Mos1 allele - chromosome II	CGC
JPS519	*vxEx519(ptl1:htauP301L:unc54, pmyo2:mch:unc54, pmyo3:mch)*	Ex htau P301L in EG4322	created for this project
JPS520	*vxEx520(ptl1:htauP301L:unc54, pmyo2:mch:unc54, pmyo3:mch)*	Ex htau P301L in EG4322	created for this project

Table 1. Four strains total were used in this project. Two strains were created for this project.

2. Materials and Methods

2.1 Strains and maintenance

C. elegans strains were maintained on nematode growth media (NGM) agar plates seeded with OP-50 *E. coli* [15]. Plates were stored at room temperature (20°C). The following strains were used: wild-type N2, and EG4322. Two new strains were developed for this work: JPS519 and JPS520. See **Table 1** for strain details.

2.2 Creation of *tau* injection constructs for MosI-mediated single-copy insertion (MosSCI)

2.2.1 Mutagenesis of WT human tau

Vectors containing wild-type (WT) human tau (*tau – 4 repeat* (*4R*)) and a non-aggregating form of human tau (*tau-3-repeat (3R)*) were acquired from a connected lab (Eriksen lab). Using the Agilent mutagenesis kit and primers designed to introduce a point mutation into WT *tau* at amino acid 301 (P301L), a mutated version of the gene encoding human tau was formed: *tau-P301L*.

2.2.2 Assembly and injection of *tau* constructs for MosSCI

Each version of *tau* (*tau-4R, tau-3R* and *tau-P301L*) was amplified through transformation of *E. coli* and Gateway overhangs were added to the gene using polymerase chain reaction (PCR). Using a BP reaction, each version of *tau* (*tau-4R, tau-3R* and *tau-P301L*) was integrated into Gateway entry vectors. The promoter for the worm homolog of tau (*ptl-1p*) was cloned from N2 whole worm lysis, PCR was used to add Gateway overhangs and the resulting product was inserted into a Gateway entry vector. A stock of Gateway entry vector containing *unc-54 UTR* (a frequently used untranslated region – UTR) was obtained. An LR reaction was performed to combine the three gene parts, creating the injection plasmids: *ptl-1p::tau::unc-54*. MosSCI *tau* injection mixtures were prepared as described in [14] Frøkjær-Jensen *et al.* (2008) and injected into EG4322 worms.

2.3 Characterization Assays

2.3.1 Head-bend assay

Worms were staged by bleaching. Worms were moved (ten at a time) to an agar plate free of OP-50. The number of head-bends (complete left-right-left or right-left-right movement) made in 10 seconds while moving forward was counted for each worm. Each worm was removed after counting head-bends to avoid re-counting. Each set of ten worms was given ten minutes total for each worm to accomplish forward movement for 10 seconds. Worms that failed to move in the given ten minutes were given a count of "0" head bends.

2.3.2 Liquid thrashing assay

Worms were staged by bleaching. Worms were moved (ten at a time) into a 10ul puddle of M9 buffer (3g KH_2PO_4, 6g Na_2HPO_4, 5g NaCl, 1mL 1M $MgSO_4$, H2O to 1 liter [15]) on an agar plate free of OP-50. M9 was refreshed as needed. Worms were allowed to settle for 10-15 seconds. Thrashing movement was recorded for 1.5-2 minutes using StreamPix 3 software (20-30 fps). A segment of 10 seconds duration within the first minute of movement was chosen for analysis from each trial video. The percentage of worms able to accomplish at least one proper C-shaped bend during the 10-second video segment was counted.

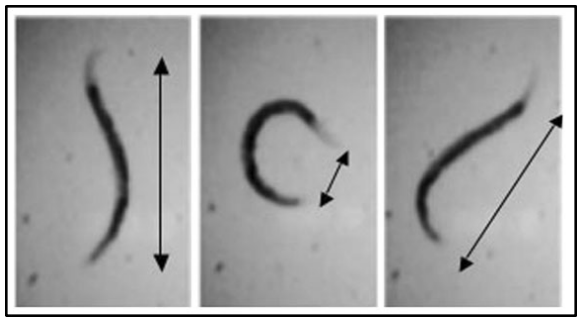

Figure 3. Proper C-shaped bend made by *C. elegans* while swimming (image obtained from http://www.wormbook.org/chapters/www_behavior/behavior.html)

2.3.3 Egg-laying assay

Worms were staged by bleaching. Ten day 1 adults were moved to a new NGM agar plate seeded with OP50 and left on the plate for a set number of hours (7 hours). Worms were removed from the plate after seven hours, and the number of eggs laid on the plate was counted.

3. Results

C. elegans successfully incorporated *ptl-1::tau-P301L::unc54 UTR* into an extrachromosomal array (JPS519 and JPS520). No other injections were successful and no injections yielded successful single-copy insertions. Transformants with potentially successful single copy insertions of *tau-P301L* and *tau4R* died without producing progeny. Due to the absence of other successful injections, JPS519 and JPS520 phenotypes were assessed using N2s as a control.

3.1 Worms expressing extrachromosomal mutant *tau-P301L* demonstrate lower performance on behavioral assays

As seen previously using a pan-neuronal promoter [7], transgenic worms expressing an extra-chromosomal array of *tau-P301L* driven by the promoter for the worm homolog (*ptl-1*) demonstrated reduced locomotive ability. Assessing locomotion on land, JPS519 and JPS520 showed less movement, characterized by a significantly decreased number of head-bends relative to N2 controls (Figure 4). (2-tailed t-test, $p<0.001$ for both JPS519 and JPS520).

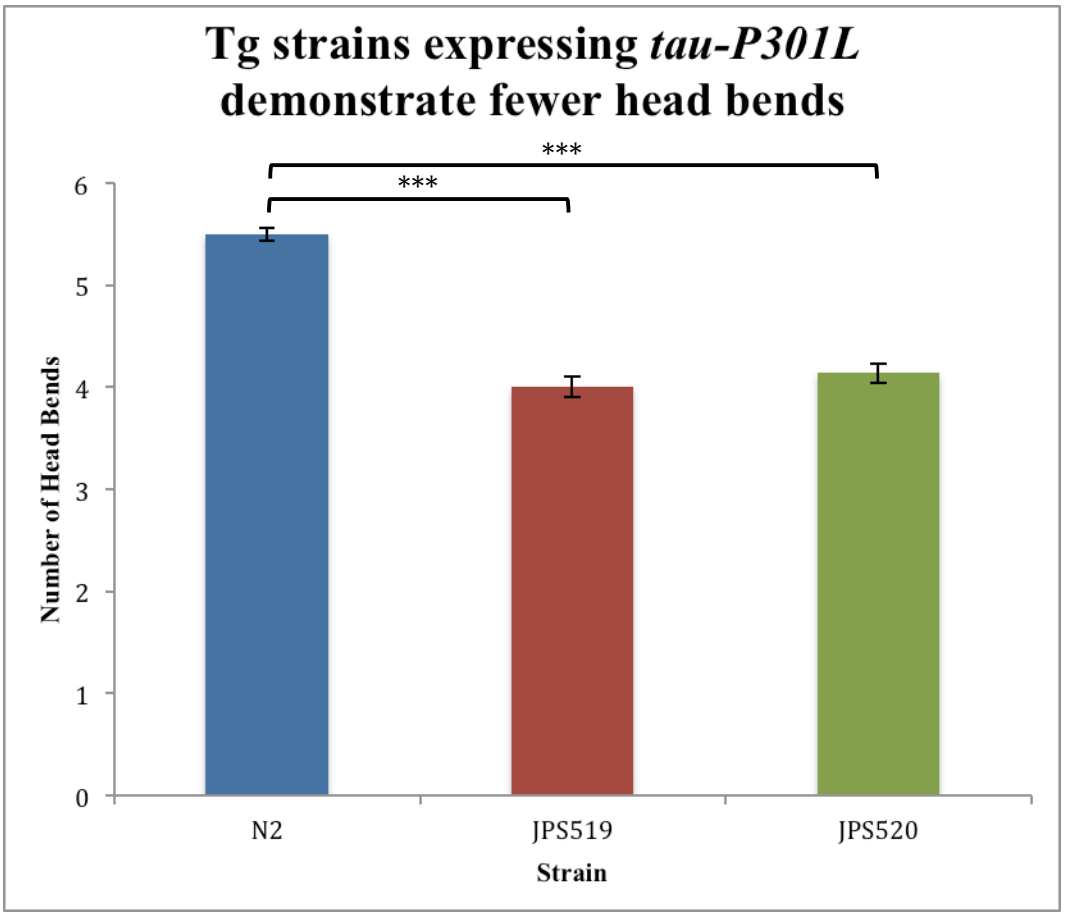

Figure 4. The number of head-bends accomplished (within 10-seconds of forward movement on an un-seeded NGM agar plate) trended toward significance, with Tg strains making fewer head-bends (***p-value<0.0001).

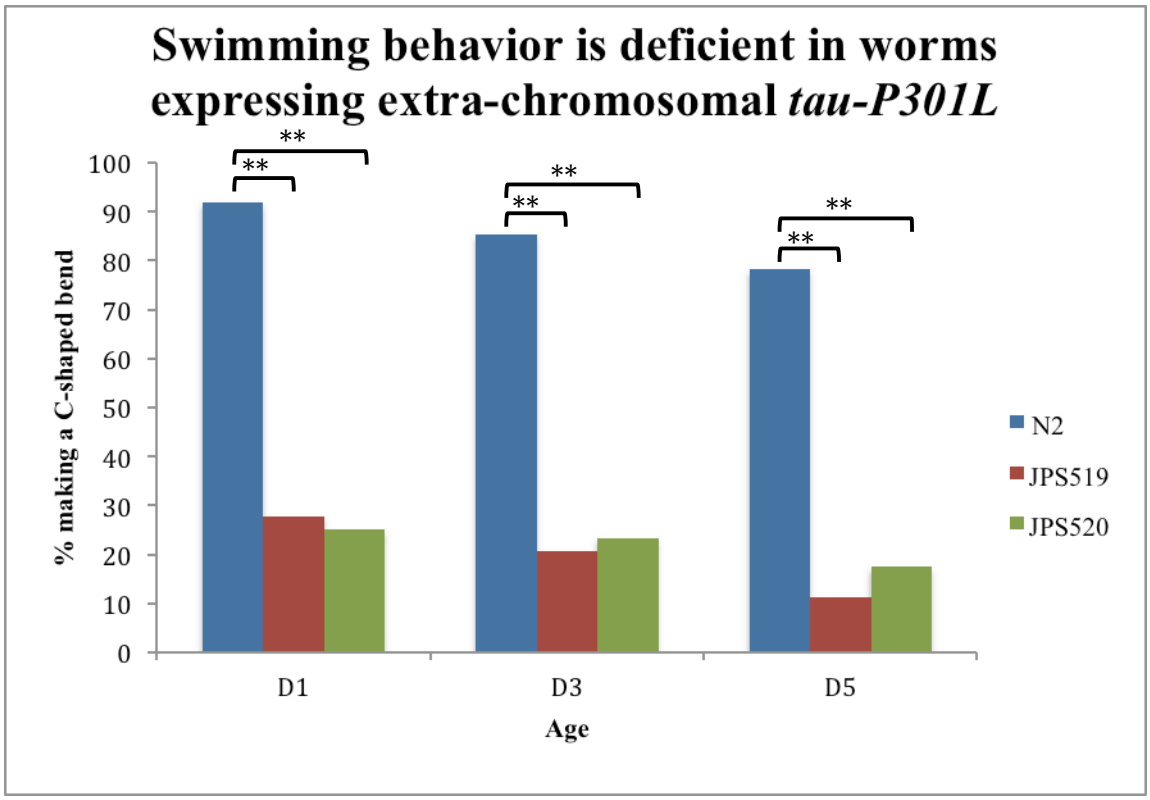

Figure 5. The percentage of worms able to make a proper C-shaped bend while swimming was significantly lower in strains expressing extra-chromosomal *tau-P301L*. JPS519 and JPS520 data was compared to same-day N2s for each situation. (***p-value=<0.0001)

Locomotive deficits were better observed in liquid thrashing assays, where *tau-P301L*-expressing worms performed significantly worse than N2s (using Fisher's exact test and comparing Tg data to same-day N2s, all p-values <0.0001). A significantly smaller percentage of worms were able to complete a C-shaped bend within a 10-second period in the strains expressing extra-chromosomal mutant *tau-P301L* (Figure 5). Though this effect seemed to have a slight tendency to worsen with age, this did not appear to be substantial.

Transgenic worms were also observed to tend to lay fewer eggs. Although only three trials of the egg-laying assays were accomplished, strains expressing extra-chromosomal *tau-P301L* (JPS519 and JPS520) appeared to lay fewer eggs than wild-type N2s (2-tailed t-test comparing Tg strains to N2 –JPS519: p=0.12; JPS520: p=0.053; Figure 6). However, there was not sufficient power to establish a significant difference.

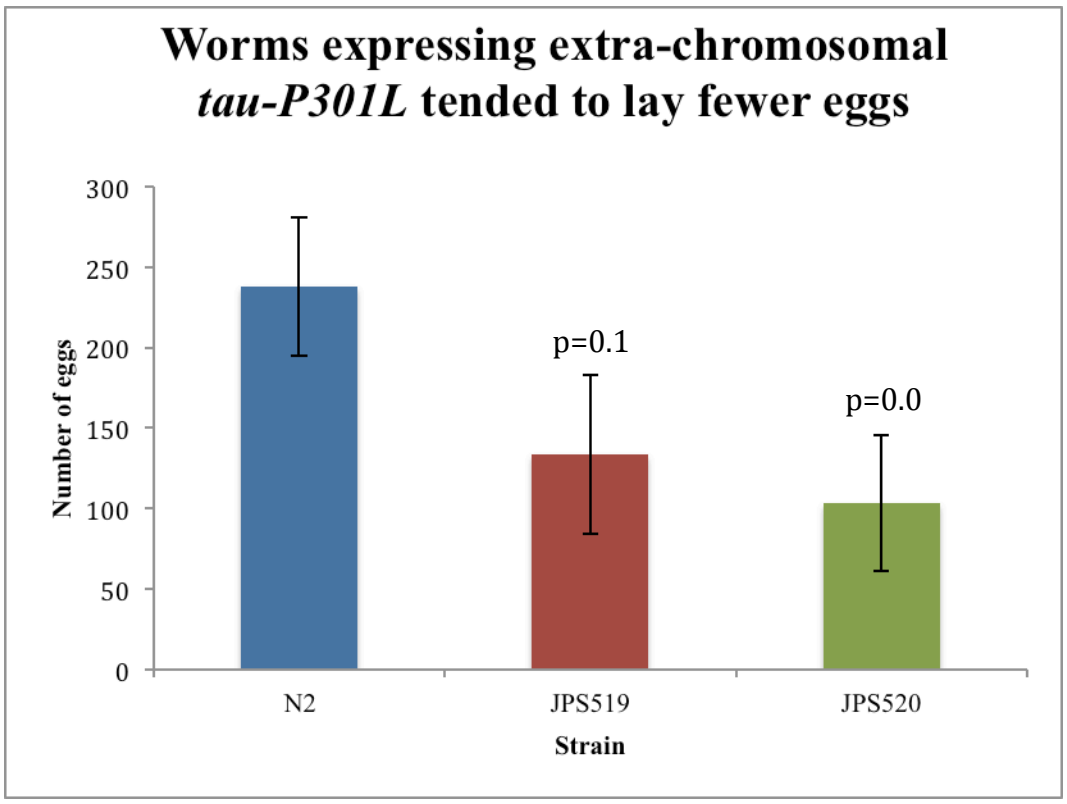

Figure 6. Tg strains expressing extra-chromosomal *tau-P301L* laid fewer eggs on a seeded NGM agar plate than wild-type N2 controls after a period of 7 hours.

Although time did not allow for more specific assessments, general observations regarding the phenotype were also made. The head-bend and liquid-thrashing assays demonstrated impaired movement, and in conducting these assays, there appeared to be an amount of tail-dragging among transgenic worms that increased with age. Additionally, although no direct assessment of life-span was made, it was noted that in spite of moving similar numbers of worms from each strain (N2, JPS519 and JPS520) onto new plates for day 1, day 3 and day 5 swimming assessment, fewer worms from the transgenic strains relative to N2 appeared to have survived to day 5. In essence, although the transgenic worms appeared normal up through early adulthood, they showed impaired locomotion, reduced egg-laying and seemingly shortened life-spans.

4. Discussion

This study was designed and carried out with the goal of creating a new model of tauopathy.

4.1 Extra-chromosomal mutant tau causes behavioral deficits

I explored whether a model of tauopathy could be made using endogenously-restricted locations of expression. Worms with mutant tau under the endogenous promoter showed fewer head bends in forward movement, made fewer proper C-shaped bends while swimming and trended toward laying fewer eggs. These worms also appeared to have shorter lifespans, though this was not quantitatively assessed. Similar motor deficits were found with a previous tau model using a pan-neuronal promoter. A strain with a non-mutant form of tau would help to determine if mutant tau is required for behavioral defects or overexpression of wild-type tau is sufficient. Kraemer *et al.* (2003) created a model expressing wild-type tau under a pan-neuronal promoter. Although, this model demonstrated behavioral deficits, defects were not as severe as in the mutant tau models, suggesting the mutant form of tau may be causing the severity of the observed phenotypes. Together these findings and previous studies show that both pan-neuronal and endogenous overexpression of mutant tau cause similar deleterious behavioral effects. The current model was created with the intent to create a single-copy integrated *tau* worm model of tauopathy. Unlike the previous pan-neuronally driven P301L tau model, the expression of *tau-P301L* in the current model was driven by the promoter for *ptl-1*, resulting in expression in the embryonic epidermis and in the mechanosensory neurons [16]. Up through adulthood, developing worms appeared to move normally, which contrasts with the results seen in [7] Kraemer *et al*., 2003, where uncoordinated movement was often seen after hatching. Since [7] Kraemer *et al*., 2003, used a pan-neuronal promoter while the current model used a less-broadly expressed promoter, this suggests that the quantity and/or specific location of tau expression may be causing the behavioral changes. A single copy insertion of tau will help determine whether there is a dose-dependent effect of tau overexpression.

In the present study, I created a new tauopathy model expressing an endogenously-driven mutant form of tau (*tau-P301L)*, which demonstrated behavioral deficits. Further exploration is needed to more accurately and thoroughly characterize this model. Additional injections must be done to acquire a single-copy integrated model of tauopathy to explore whether phenotypic differences between this model and previous models [7] are dose or location-dependent.

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